

REVIEW PAPER

# Roles of melatonin in abiotic stress resistance in plants

Na Zhang<sup>1,\*</sup>, Qianqian Sun<sup>1,\*</sup>, Haijun Zhang<sup>1,\*</sup>, Yunyun Cao<sup>1</sup>, Sarah Weeda<sup>2</sup>, Shuxin Ren<sup>2,†</sup> and Yang-Dong Guo<sup>1,†</sup>

<sup>1</sup> College of Agriculture and Biotechnology, China Agricultural University, Beijing, China

<sup>2</sup> School of Agriculture, Virginia State University, Petersburg, VA, USA

\* These authors contributed equally to this work.

† To whom correspondence should be addressed. E-mail: [yaguo@cau.edu.cn](mailto:yaguo@cau.edu.cn) and [sren@vsu.edu](mailto:sren@vsu.edu)

Received 6 June 2014; Revised 11 July 2014; Accepted 13 July 2014

## Abstract

In recent years melatonin has emerged as a research highlight in plant studies. Melatonin has different functions in many aspects of plant growth and development. The most frequently mentioned functions of melatonin are related to abiotic stresses such as drought, radiation, extreme temperature, and chemical stresses. This review mainly focuses on the regulatory effects of melatonin when plants face harsh environmental conditions. Evidence indicates that environmental stress can increase the level of endogenous melatonin in plants. Overexpression of the melatonin biosynthetic genes elevates melatonin levels in transgenic plants. The transgenic plants show enhanced tolerance to abiotic stresses. Exogenously applied melatonin can also improve the ability of plants to tolerate abiotic stresses. The mechanisms by which melatonin alleviates abiotic stresses are discussed.

**Key words:** Abiotic stress, genetic modification, mechanism, melatonin, plants, tolerance.

## Introduction

Melatonin is an indoleamine synthesized throughout the plant kingdom. It is amphiphilic, which enables it to cross cell membranes easily and enter subcellular compartments (Shida *et al.*, 1994). Melatonin was named as such due to its ability to lighten skin in certain fish, reptiles, and amphibians (Lerner *et al.*, 1958). In vertebrates, melatonin is mostly known as a biological modulator of mood, sleep, sexual behaviour, seasonal reproductive physiology, circadian rhythms, and immunology (Arnao and Hernández-Ruiz, 2006; Reiter *et al.*, 2010; Cipolla-Neto *et al.*, 2014). In plants, research efforts over the past decade have focused on determining its many roles in plant physiology.

Solid evidence implicates melatonin as a growth promoter and rooting agent (Hernández-Ruiz *et al.*, 2004, 2005; Arnao and Hernández-Ruiz, 2007; Hernández-Ruiz and Arnao, 2008; Chen *et al.*, 2009; Sarrou *et al.*, 2014; Zhang *et al.*, 2014). In addition to its roles in plant development, melatonin plays an important role in plant stress defence. Plants may frequently encounter stressful environmental conditions. Various plant

species rich in melatonin have shown a higher capacity for stress tolerance. Exogenous treatment or ectopic overexpression of melatonin biosynthetic genes can also improve the resistance against a series of stressors, including extreme temperature, drought, salinity, radiation, and chemical stresses, all of which lead to reactive oxygen species (ROS) generation. Melatonin is also involved in stress-affected developmental transitions, including flowering, fruiting, and senescence. To understand these phenomena and in order to take full advantage of this molecule in agriculture, we must determine the reason for the existence of melatonin in plants and the biochemical mechanisms in response to external stimuli.

We begin this review by emphasizing the roles of melatonin and its contribution to alleviate abiotic stress in plants. Various abiotic stresses inhibit plant growth via different mechanisms, but all cause a rise in ROS levels, and disrupt the ROS homeostasis. The mechanisms of the involvement of melatonin in modulating stress resistance are summarized herein.

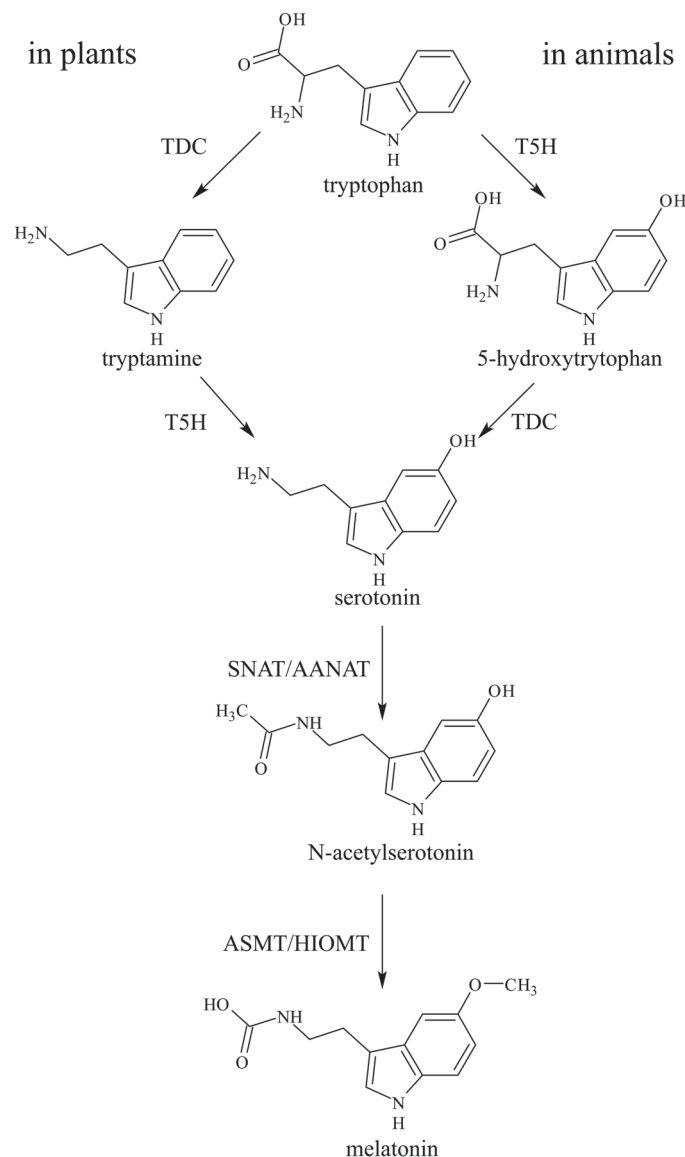
## Melatonin biosynthesis pathway in plants

Isotope tracer experiments have confirmed that melatonin is synthesized in plants (Murch *et al.*, 2000). It has been suggested that plant melatonin is synthesized via similar biosynthetic pathways to those in animals (Fig. 1). Additionally, the enzymes which take part in melatonin biosynthesis have been cloned. Melatonin biosynthesis in plants occurs via four consecutive enzymatic steps (Kang *et al.*, 2011). The first committed enzyme is tryptophan decarboxylase (TDC), which catalyses the conversion of tryptophan into tryptamine. *TDC* has been cloned in several plant species including rice (Kang *et al.*, 2008), pepper (Park *et al.*, 2009), *Catharanthus roseus* (De luca *et al.*, 1989), and tobacco (Di Fiore *et al.*, 2002). *TDC* serves as a bottleneck in regulating serotonin (precursor

of melatonin) biosynthesis since *TDC* expression is very low or negligible.

The second step in melatonin biosynthesis is catalysed by the cytochrome P450 enzyme tryptamine 5-hydroxylase (*T5H*), which hydroxylates the C-5 position of tryptamine to form serotonin (Fujiwara *et al.*, 2010). *T5H* has been isolated in rice sekiguchi lesion (*sl*) mutants by map-based cloning (Fujiwara *et al.*, 2010). *T5H* is constitutively expressed in healthy rice (*Oryza sativa*) plants (Kang *et al.*, 2007).

The final two enzymes on the melatonin biosynthetic pathway are arylalkylamine *N*-acetyltransferase (*AANAT*) and *N*-acetylserotonin methyltransferase (*ASMT*). Currently, there are no known *AANAT* homologous genes in higher plants; however, *AANAT* has been cloned in the unicellular green alga *Chlamydomonas reinhardtii* (Okazaki *et al.*, 2009). Kang *et al.* have cloned rice serotonin *N*-acetyltransferase (*SNAT*) genes, which encode an enzyme catalysing conversion of serotonin into *N*-acetylserotonin. The last enzyme, *ASMT*, which was known as *HIOMT* (hydroxyindole-*O*-methyltransferase), has been purified in rice via heterologous *Escherichia coli* overexpression (Kang *et al.*, 2013). *ASMT* is the rate-limiting enzyme of melatonin biosynthesis. Subcellular localization shows that *SNAT* protein is localized in chloroplasts, whereas *ASMT* is found in the cytoplasm (Byeon *et al.*, 2014a).



**Fig. 1.** Biosynthetic pathway of melatonin in plants and in animals. TDC, tryptophan decarboxylase; T5H, tryptamine 5-hydroxylase; SNAT, serotonin *N*-acetyltransferase; AANAT, arylalkylamine *N*-acetyltransferase; ASMT, *N*-acetylserotonin methyltransferase; HIOMT, hydroxyindole-*O*-methyltransferase.

## Genetic modification changes stress tolerance in plants

The first melatonin biosynthesis gene, *TDC*, has been overexpressed in rice (Byeon *et al.*, 2014b). Melatonin accumulation is seed specific in *TDC3* transgenic lines; transgenic seeds exhibited melatonin concentrations 31-fold higher than those in wild-type seeds. The level of melatonin intermediates also increased in both homologous and ectopic *TDC* overexpression lines (Kang *et al.*, 2008; Kanjanaphachot *et al.*, 2012). Transgenic rice plants that overexpress *TDC* exhibit delayed senescence of leaves (Kang *et al.*, 2009), while suppression of *TDC* by RNA interference (RNAi) produced lower serotonin levels and promoted the senescence process. Fungal infection of pepper fruit led to an increase of *TDC* gene expression (Park *et al.*, 2009), indicating that melatonin is responsive to pathogen attack. Rice roots maintain high *T5H* activity, and *T5H* expression can be induced by *Magnaporthe grisea* infection, the causal pathogen of rice blast disease. Exogenously applied serotonin, the penultimate substrate for melatonin biosynthesis, increased resistance to rice blast infection in plants (Fujiwara *et al.*, 2010). Exogenously applied melatonin improved resistance to Marssonina apple blotch (*Diplocarpon mali*) which is one of the most serious diseases leading to defoliation during the growth season (Yin *et al.*, 2013). This also implies that melatonin plays an important role in plant innate immunity.

An *AANAT* gene homologue is absent in the plant genome. Okazaki *et al.* (2009) have isolated an *AANAT* from the unicellular green alga *C. reinhardtii* and introduced it into the Micro-Tom tomato genome. Its ectopic overexpression in tomato successfully resulted in higher melatonin content. In

rice plants, *SNAT* is substituted for *AANAT* to encode the gene for the penultimate enzyme in plant melatonin biosynthesis (Kang *et al.*, 2013). Transgenic rice expressing the human serotonin *N*-acetyltransferase gene (*SNA/AANAT*) exhibited high levels of melatonin and elevated chlorophyll synthesis during cold stress, suggesting that melatonin plays a role in cold stress tolerance (Kang *et al.*, 2010). Transgenic rice seedlings expressing ovine *AANAT* display enhanced seminal root elongation (Park and Back, 2012), seedling growth (Byeon and Back, 2014), and resistance to herbicide-induced oxidative stress (Park *et al.*, 2013b). Microarray analysis of this transgenic line clearly demonstrated that the senescence-associated proteins are significantly down-regulated (Byeon *et al.*, 2013). High temperatures and dark conditions increased melatonin levels by increasing the activities of the final two enzymes in melatonin synthesis (Byeon and Back, 2013). This implies a role for melatonin in defence against high temperature. Transgenic Micro-Tom tomato plants overexpressing the homologous ovine *AANAT* and *HIOMT* genes display loss of apical dominance and enhanced drought tolerance (Wang *et al.*, 2014). Furthermore, rice *ASMT* mRNA can be induced upon abscisic acid (ABA) and methyl jasmonic acid treatments, suggesting the potential involvement of melatonin in response to various stresses (Park *et al.*, 2013a). Transgenic plants of *Nicotiana sylvestris* expressing an *AANAT* gene and a *HIOMT* gene have enhanced production of melatonin. When exposed to ultraviolet (UV)-B radiation, melatonin exhibits a protective effect against UV-B-induced DNA damage (Zhang *et al.*, 2012).

## Melatonin levels in plants under stress conditions

It is widely reported that the melatonin concentration inside the cell is responsive to external conditions. A rapid change in light, temperature, and various environmental stress agents may increase melatonin levels. The level of melatonin in plants is also variable under different environmental conditions, especially under stress. Arnao and Hernández-Ruiz determined the melatonin level in barley roots (Arnao and Hernández-Ruiz, 2009b) and lupin (Arnao and Hernández-Ruiz, 2013) under natural or artificially induced adverse conditions. The former experiment treated plants with the chemical stress agents sodium chloride, zinc sulphate, or hydrogen peroxide. Drought, anaerobic, pH, and cold stress were included in the latter experiment. However, all chemical stresses produced similar changes in melatonin levels. Low temperature and drought cause pronounced changes in the endogenous level of melatonin following the chemical stress  $ZnSO_4$  or NaCl. Restricting the oxygen supply to roots also caused a slight increase in root melatonin levels. Under pH stress, alkalization of the medium lowered the root melatonin content, whereas acidification resulted in a slight increase. Additionally, the responses in both lupin and barley were clearly dose and time dependent. The authors concluded that nearly all stress factors can cause an up-regulation in melatonin biosynthesis in the investigated plants.

Environmental conditions decisively affect the melatonin content of tissues. Plants grown indoors, in moderate conditions, have lower melatonin content than those cultivated in the field with more variable conditions. Plants grown in sunlight possess three times more melatonin in roots and 2.5 times more in leaves compared with those grown under artificial light (Tan *et al.*, 2007a). In pepper fruits, the melatonin content of shaded fruit is lower than that of non-shaded fruit, which indicates that solar radiation causes a rise in melatonin levels (Riga *et al.*, 2014). The melatonin levels in *Glycyrrhiza uralensis* under lights of different spectral quality have also been examined. Melatonin levels in the roots can be enhanced when plants are exposed to UV-B irradiation. The authors speculate that the elevated melatonin production under stress may be an adaptive reaction of plants to tolerate adverse environmental conditions (Afreen *et al.*, 2006). Furthermore, plants exhibiting different susceptibilities to ozone injury vary in their melatonin content. Plant species which are more resistant to ozone damage maintain higher melatonin levels than sensitive species (Dubbels *et al.*, 1995). Alpine and Mediterranean plants exposed to high UV in their natural habitat contain much higher melatonin levels than the same species living under lower UV exposure (Simopoulos *et al.*, 2005). Therefore, a key role for melatonin may relate to the processes of photosynthesis or photoprotection.

Melatonin and its metabolite *N*<sup>1</sup>-acetyl-*N*<sup>2</sup>-formyl-5-methoxykynuramine (AFMK) are present in the pollutant-tolerant plant, water hyacinth. Studies have shown that the hyacinth can tolerate contamination of wastewater generated from industrial and agricultural sources (Trivedy and Pattanshetty, 2002; Singhal and Rai, 2003; Munavalli and Saler, 2009), such as nitrogen and phosphorus (Jayaweera and Kasturiarachchi, 2004), the pesticide ethion (Xia and Ma, 2006), the heavy metal mercury (Riddle *et al.*, 2002), and the carcinogen arsenic (Misbahuddin and Fariduddin, 2002). Artificially supplemented melatonin can make this plant even more useful for phytoremediation, because it is a powerful antioxidant capable of elevating their tolerance to pollutants and removing additional contaminants. Since water hyacinth has high levels of melatonin and AFMK, we hypothesize that the elevated concentration of these natural antioxidants may be associated with the high tolerance of this plant to pollutants and to its utility in phytoremediation.

Rice seedlings at high temperatures and under dark conditions show enhanced melatonin synthesis due to increased serotonin *N*-acetyltransferase and *N*-acetylserotonin methyltransferase activities (Byeon and Back, 2013). In green microalgae *Ulva* sp., temperature and the heavy metals cadmium, lead, and zinc induced a rise in melatonin levels (Tal *et al.*, 2011). Exposure to cadmium induces a significant rise in melatonin levels in the algae, while lead and zinc exposure also induced an increase in melatonin levels, but to a lesser extent. This suggests that melatonin participates in adaptation to environmental stress.

A high content of melatonin may also play a protective role in seeds during sprouting. In plants, the melatonin levels vary substantially among organs, from picograms to micrograms per gram of tissue (Chen *et al.*, 2003; Arnao and

Hernández-Ruiz, 2006, 2009a; Hernández-Ruiz and Arnao, 2008; Paredes *et al.*, 2009; Posmyk and Janas, 2009), with the highest levels detected in reproductive organs, particularly in seeds. In sprouting sunflower seeds, an increase in melatonin content was detected (Cho *et al.*, 2008). Since the germ tissue is highly vulnerable to oxidative damage, we surmise that melatonin might be present as an important component of its antioxidant defence system as a free radical scavenger. Thus, melatonin in seeds may be essential for protecting germ tissue and reproductive tissue of plants from oxidative damage (Manchester *et al.*, 2000).

Melatonin levels in plants increase considerably under various stress conditions. Such an increase is believed to be beneficial for stress resistance. Exogenous application of melatonin is often implicated as a means of ameliorating the detrimental effects of stress.

### Exogenously applied melatonin affects stress tolerance in plants

The cloned genes of melatonin synthesis enzymes prove that the plants have the necessary enzymatic machinery for melatonin biosynthesis. In addition to *in vivo* synthesis, plants can also absorb exogenously provided melatonin from the environment and accumulate it in their organs (Tan *et al.*, 2007a). Tritium-labelled melatonin was readily taken up by *Chenopodium rubrum* seedlings and remained stable 37 h after application (Kolar *et al.*, 2003).

The effect of exogenously applied melatonin ranges from a significant amelioration to being ineffective or even toxic. The concentration is the cause of the difference. Melatonin may play significantly different roles in regulating plant growth and development under low and high concentrations in the same species. In the case of wild leaf mustard (*Brassica juncea*), a low level of melatonin (0.1 mM) stimulated root growth, while a high level (100 mM) inhibited growth (Chen *et al.*, 2009). Additionally, melatonin promotes rooting at a low concentration but inhibits growth at high concentrations in cherry tissue culture (Sarropoulou *et al.*, 2012a). Since melatonin levels in plants vary from picograms to micrograms per gram of tissue, the high concentrations used, namely 100 mM, would never be achievable in any plant. Indeed, this is many orders of magnitude above physiological concentrations. Concentrations which are too high can cause toxic effects. In the analysis of gene expression modulated by melatonin, it was found that low and high levels of melatonin had a differential effect. Not all genes regulated by low melatonin were regulated by high melatonin (Weeda *et al.*, 2014). This means that melatonin may work in different ways at low and high concentrations. High concentrations of melatonin may severely reduce ROS in cells, thereby affecting ROS-dependent signal transduction and inhibiting cell growth (Afreen *et al.*, 2006). Plant species vary in their sensitivity to melatonin. Melatonin has an effect on lupin and barley root physiology. However, lupin roots are more sensitive to melatonin than barley, since the responses in lupin roots need 24 h, while barley roots need 72 h to show a similar response.

Roots directly influence the stress resistance of plants. Roots are very sensitive to changes in pH, salinity, oxygen availability, toxic elements, and water potential in their rhizosphere. A strong root system helps resist the adverse effects of stress. The function of melatonin as a rooting agent has been demonstrated many times. For example, melatonin has an effect on the regeneration of lateral and adventitious roots in etiolated hypocotyls of *Lupin albus* and *B. juncea* (Arnao and Hernández-Ruiz, 2007; Chen *et al.*, 2009). Melatonin induced the appearance of root primordia from pericycle cells, modified the distribution pattern of adventitious or lateral roots, and affected the timing of adventitious root formation, the number and length of adventitious roots, and the number of lateral roots. Melatonin also modulates *Arabidopsis* root system architecture by stimulating lateral and adventitious root formation (Pelagio-Flores *et al.*, 2012; Koyama *et al.*, 2013). Transgenic rice seedlings expressing ovine *SNAT*, which is believed to be the rate-limiting enzyme in melatonin biosynthesis, exhibits enhanced seminal root growth (Park and Back, 2012). In tissue culture systems, melatonin also has a function in root regeneration. The explants used in these experiments were mostly from woody plant species (Murch *et al.*, 2001; Sarropoulou *et al.*, 2012b; Sarrou *et al.*, 2014). An increase in the endogenous concentration of melatonin correlated with an increase in *de novo* root formation in St. John's wort (*Hypericum perforatum* cv. Anthos) (Murch *et al.*, 2001). It is well documented that the control of cellular redox homeostasis is highly related to root development (Passaia *et al.*, 2014). The root is often implicated as the potential site for melatonin biosynthesis due to the high melatonin levels found therein. Furthermore, there is a clear concentration gradient of melatonin in lupin roots, between apical and basal zones and between the main (primary) and lateral (secondary) roots (Arnao and Hernández-Ruiz, 2007; Hernández-Ruiz and Arnao, 2008). Generally, the highest melatonin concentration is found in the most actively growing zones.

Under high salinity and drought conditions, plants have reduced growth and a marked decline in their net photosynthetic rates and chlorophyll content. Water stress also causes an inhibition of seed germination and root vitality. However, pre-treatment with melatonin significantly reverses this inhibition (Li *et al.*, 2012; Zhang *et al.*, 2013, 2014). This is demonstrated by comparing reductions in shoot height, leaf numbers, root systems, photosynthetic capacity, and biomass production. Pre-treatment with melatonin significantly alleviates this growth inhibition, thus enabling plants to maintain a robust root system and improve photosynthetic capacity.

Exposure to low temperature triggers biochemical and physiological changes in plants and causes a loss of vigour and reduced growth rate. Cell membrane systems are the primary sites of chilling injuries. Low temperature leads to changes in cell structure, cell membranes, and cell wall composition (Kratsch and Wise, 2000). The effect of chilling stress depends on the degree of severity and the time of exposure. The protective effect of exogenous melatonin during chilling stress has been described. Mung bean (*Vigna radiata* L.), a plant originating from the tropics, is highly vulnerable to chilling (Hung *et al.*, 2007). Three-day-old seedlings were

exposed to 5 °C for 2 d then returned to optimal temperature (25 °C). The seedlings from seeds primed with melatonin showed a 20% increase in root length and had less disorganized cell ultrastructure (Szafranska *et al.*, 2013). Similar effects of melatonin have also been found in cucumber under osmotic stress (Zhang *et al.*, 2013). Melatonin-treated mung beans also had an increased accumulation of total phenolic compounds and proline (Szafranska *et al.*, 2012). Cucumber seeds treated with melatonin exhibit an improved germination rate during chilling stress (Posmyk *et al.*, 2009). Plant growth data indicate that *Arabidopsis* plants treated with low concentrations of melatonin (10 µM and 20 µM) show enhanced primary root growth and seedling fresh weight. Pre-treatment with melatonin attenuated apoptosis induced by cold temperature in cultured carrot suspension cells (Lei *et al.*, 2004). Cold stress-induced shrinkage and disruption of carrot cell plasma membranes were almost completely alleviated by melatonin treatment. This suggests that melatonin is helpful in coping with harsh environments by maintaining membrane integrity. Lupin plants grown in a cold environment (6 °C) show a 2.5-fold increase in melatonin levels compared with control plants grown at 24 °C. Cryopreservation is a process during which cells or tissues are cooled to sub-zero temperatures. During cryopreservation, environmental changes including osmotic injury, desiccation, and low temperature can impose a series of stresses on plants (Zhao *et al.*, 2011). The survival rate of the cryopreserved *Rhodiola crenulata* callus is ~60%. When the callus was pre-treated with 0.1 µM melatonin prior to freezing in liquid nitrogen, the survival rate significantly increased. Melatonin significantly enhanced the recovery of cryopreserved shoot tips of American elm (*Ulmus americana* L.) (Uchendu *et al.*, 2013). Shoot explants grown in melatonin-enriched media showed increased regrowth. Melatonin can significantly reverse the inhibitory effects of light and high temperature on germination of photosensitive and thermosensitive *Phacelia tanacetifolia* Benth seeds (Tiryaki and Keles, 2012).

Melatonin delays drought- and dark-induced leaf senescence in apple (Wang *et al.*, 2012, 2013a, b). Melatonin helped maintain photosystem II (PSII) function under stress and delayed the typical reduction in chlorophyll content. Detached leaves treated with 10 mmol l<sup>-1</sup> melatonin showed noticeably slower dark-induced senescence. Melatonin treatment can suppress the up-regulation of senescence-associated gene 12 (*SAG12*), a marker gene for senescence during dark-induced senescence, implicating that melatonin can inhibit senescence. In barley leaves treated with melatonin solutions, there is a clear slowing of chlorophyll loss in leaves, which is one of the main processes that occurs during leaf senescence (Arnao and Hernández-Ruiz, 2009c). Melatonin can also alleviate paraquat-induced photobleaching. Leaves treated with paraquat in the absence of melatonin became completely photobleached, while leaves treated with 1 mM melatonin remained green, similar to leaves in the absence of paraquat (Weeda *et al.*, 2014). Rice leaves subjected to senescence treatment exhibit enhanced endogenous melatonin levels (Byeon *et al.*, 2012). Also, in melatonin-rich transgenic rice lines, senescence-associated proteins were significantly

down-regulated (Byeon *et al.*, 2013). To sum up, melatonin is closely related to the delay of senescence.

In animals, the antioxidant effects of melatonin on various oxidative stresses, such as cadmium and nickel, have been extensively investigated (Wang *et al.*, 2011). Previous studies showed that melatonin treatment might be effective in modulating some neurotoxic effects induced by metals. In the field of medicine, melatonin has been recognized in the prevention of and clinical recovery from heavy metal intoxication (Flora *et al.*, 2013). In green algae, exogenous melatonin can relieve cadmium-induced stress (Tal *et al.*, 2011). High levels of copper contamination are toxic to pea plants; however, melatonin added to the soil significantly enhanced their tolerance to copper contamination and increased their survival rate (Tan *et al.*, 2007b). Melatonin treatment also improved seed germination and seedling growth of cabbage in the presence of CuSO<sub>4</sub> (Posmyk *et al.*, 2008).

### Mechanisms of melatonin-mediated stress tolerance and detoxification

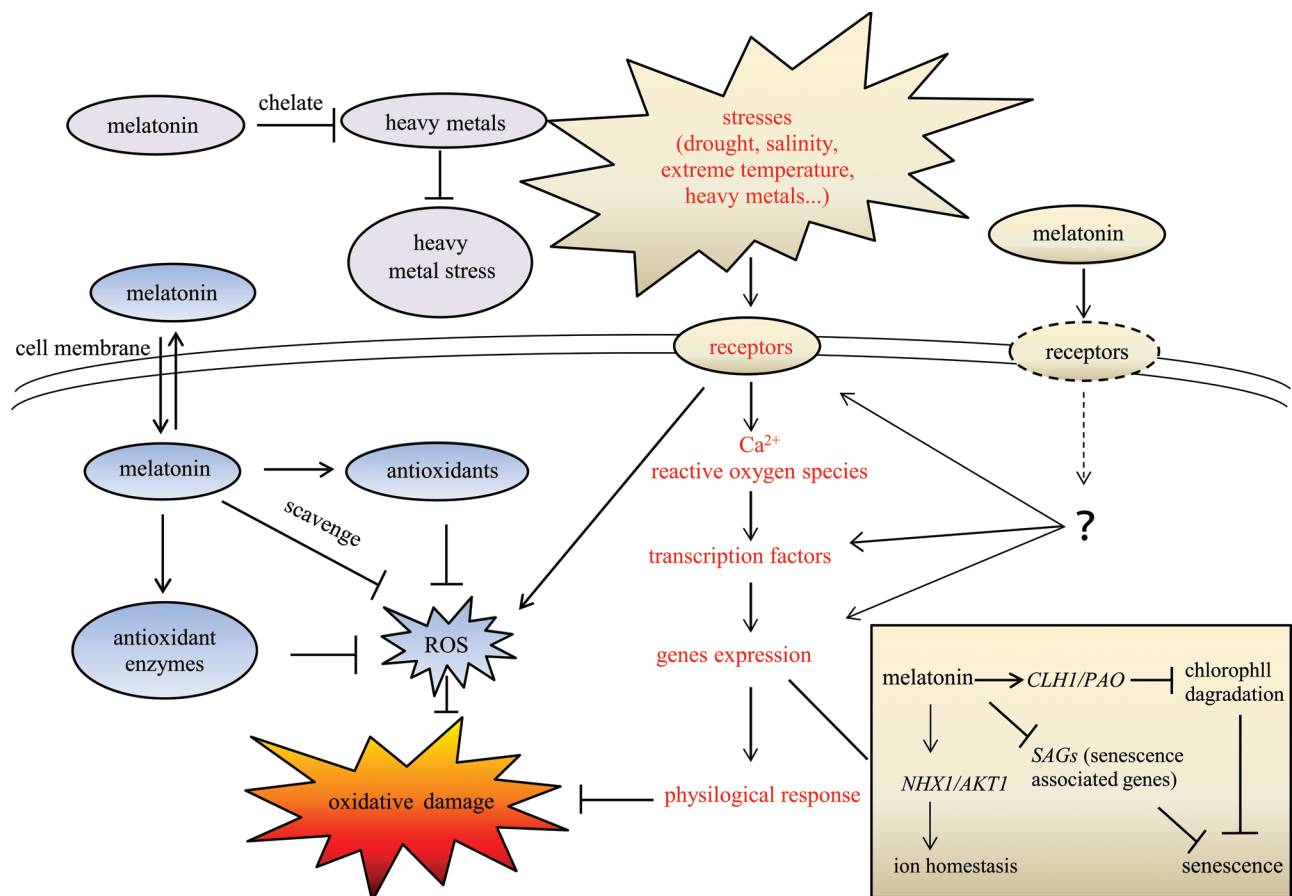
As plants are sessile, they can only adjust their own physical conditions to resist adverse environments. So when faced with a harsh environment, a rapid and tremendous change must occur inside the plant cells in order to survive. A cell is separated from its surrounding environment by a physical barrier, namely the plasma membrane. This membrane is selectively permeable to small molecules and ions. Melatonin is an amphipathic molecule which can easily diffuse through cell membranes into the cytoplasm and enter subcellular compartments.

Drought limits plant growth due to photosynthetic decline. Salinity interferes with plant growth as it leads to physiological drought and ion toxicity. Extreme temperatures impair plant growth or even cause death by affecting membrane fluidity and enzyme activity. Radiation stress can damage macromolecules, such as DNA and proteins, generate ROS, and impair cellular processes. Heavy metals such as copper and zinc are essential for normal plant growth, but excess heavy metals are toxic. The metal ions bind to sulphhydryl groups in enzymes and other proteins, thus inhibiting their activity or disrupting their structure (Hall, 2002). In addition, heavy metals cause oxidative deterioration of biomolecules by initiating free radical-mediated chain reactions resulting in lipid peroxidation, protein oxidation, and oxidation of nucleic acids. All the abiotic stresses cause an enhanced generation of ROS (Prasad *et al.*, 1994; Foyer *et al.*, 1997). While ROS are clearly required for growth and function as secondary messengers in signal transduction (Baxter *et al.*, 2014), high concentrations of ROS can trigger genetically programmed cell suicide events (Foyer and Noctor, 2005). Furthermore, excess levels of ROS can have detrimental effects as a result of their ability to cause lipid peroxidation in cellular membranes, DNA damage, protein denaturation, carbohydrate oxidation, pigment breakdown, and impaired enzyme activity (Bose *et al.*, 2014). Chloroplasts and mitochondria are a major source of ROS production in plant cells since photosynthetic and

respiratory processes generate ROS (Mittler, 2002). There is a delicate balance between ROS generation and scavenging. Major ROS-scavenging enzymes of plants include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione peroxidase (GPX). In addition to the enzymes that detoxify ROS, antioxidants such as ascorbate, glutathione (GSH), and tocopherol play an important role in the regulation of cellular ROS homeostasis.

Studies on how melatonin interacts with stress signalling mechanisms have identified a complex relationship with ROS (Fig. 2). Evidence confirms that melatonin is a broad-spectrum direct antioxidant which can scavenge ROS with high efficiency. A detailed knowledge of melatonin chemistry and molecular interactions with ROS and with strong oxidants has been documented (Tan *et al.*, 2000; Allegra *et al.*, 2003). In addition, melatonin treatments modulate the antioxidant enzymes by both up-regulating the transcript level and increasing the activity levels. Melatonin also has the capacity to enhance cellular antioxidant defence mechanisms by regenerating endogenous antioxidants, such as glutathione and vitamins C and E. They also influence cellular signalling and trigger redox-sensitive regulatory pathways. Under salinity and drought stress, the exogenous application of melatonin significantly suppressed the production of H<sub>2</sub>O<sub>2</sub> and

hydroxyl radicals (Li *et al.*, 2012; Zhang *et al.*, 2013). The transcript levels of the genes encoding SOD, APX, CAT, and peroxidase are also up-regulated by melatonin (Rodriguez *et al.*, 2004). Melatonin treatment leads to the maintenance of higher contents of ascorbic acid (AsA) and GSH (Wang *et al.*, 2012). In higher plants, the AsA–GSH cycle is an important antioxidant system protecting against oxidative stress. The theory that the addition of melatonin lessens oxidative damage caused by stress by directly scavenging ROS, and enhancing antioxidants and the activities of antioxidative enzymes has been confirmed in many experiments. Cellular responses to stress are initiated primarily by the interaction of the extracellular material with a plasma membrane protein. This means that stresses are first perceived by receptors present on the membrane of the plant cells. The signal is then transduced downstream, resulting in the generation of secondary messengers including calcium and ROS. Melatonin alters the expression of genes involved in the signal transduction steps along the way. RNA-seq analysis revealed that six stress receptors and 14 genes involved in calcium-dependent signalling are up-regulated in response to melatonin (Weeda *et al.*, 2014). Melatonin interactions with Ca<sup>2+</sup>–calmodulin have been shown to regulate many calcium-dependent cellular functions in animal cells (Posmyk and Janas, 2009). These



**Fig. 2.** Overview of cellular responses to stresses following treatment with exogenous melatonin. Melatonin chelates toxic metals in order to alleviate the heavy metal stress. Melatonin is an amphiphilic molecule which can freely cross cell membranes. Melatonin can scavenge ROS directly. It also increase the levels of antioxidants and the activities of related enzymes to scavenge ROS. Stresses are first recognized by receptors, followed by a signal transduction cascade. Melatonin alters almost all the transduction steps along the way by receptor-dependent or receptor-independent processes. Melatonin alters expression of genes related to stresses.

interactions may also occur in plants. Many transcription factors have been identified as being up-regulated by melatonin treatment, and the majority of these are stress-related transcription factors including WRKY, NAC domain-containing proteins, and zinc finger-related transcription factors. This is consistent with the study on cucumber under salinity stress (Zhang *et al.*, 2014). Many genes related to redox homeostasis were identified among the transcripts that were altered by melatonin. Most genes identified were related to biotic and/or abiotic stress responses. Gene expression of oxidative stress-alleviating enzymes including peroxidases, glutathione peroxidase, haloperoxidase, and thioredoxin reductase was induced in melatonin-rich rice and melatonin-treated plants (Byeon *et al.*, 2013; Zhang *et al.*, 2014). This implicates that melatonin may have a role in protecting important molecules such as nucleic acids, proteins, and lipids against oxidative damage.

Melatonin alters the expression of a large number of plant stress defence-related genes. In addition to the stress receptors, stress-associated calcium signals, and plant stress defence-related transcription factor genes, melatonin also regulates several specific genes related to specific stresses. For example, melatonin may preserve chlorophyll content. Melatonin significantly down-regulated the expression of chlorophyllase (CLH1), which is a light-regulated enzyme involved in chlorophyll degradation (Weeda *et al.*, 2014). This is consistent with the study conducted on melatonin affecting senescence in apple leaves. In the experiment, exogenous melatonin inhibited transcript levels of pheide a oxygenase (PAO), which is another key enzyme involved in chlorophyll degradation (Wang *et al.*, 2013b). This may provide another mechanism by which melatonin preserves chlorophyll content during senescence, in addition to attenuating ROS. These findings can explain the means by which melatonin preserves the chlorophyll content in leaves, delays senescence, and enhances photosynthetic rates. The detrimental effects of high salt result from both a water deficit caused by osmotic stress and the interference of excess sodium ions with key biochemical processes (Zhang and Blumwald, 2001). *NHX1* and *AKT1* are ion channel genes which possibly contribute to the maintenance of ion homeostasis. Expression of *NHX1* and *AKT1* is greatly up-regulated in leaves, thus improving salinity resistance in plants exposed to exogenous melatonin (Li *et al.*, 2012). Furthermore, cell death-associated genes identified were mostly down-regulated by melatonin treatment (Weeda *et al.*, 2014). This result provides another explanation for the melatonin-related antiapoptotic process. It is well established that the antiapoptotic action of melatonin in animal cells is mainly based on its antioxidant property (Lei *et al.*, 2004), whereas in the inhibition of cold-induced apoptosis by melatonin in plants, melatonin may exert its antiapoptotic action through polyamines rather than ROS attenuation. It is documented that high polyamine levels in plant cells are correlated with tolerance of a number of biotic and abiotic stresses (Bouchereau *et al.*, 1999), especially chilling injury (Kim *et al.*, 2002). Polyamines are also implicated, directly or indirectly, in pathways regulating programmed cell death (Moschou and Roubelakis-Angelakis, 2014). Melatonin up-regulates the expression of C-repeat-binding factors (CBFs)/

drought response element-binding factors (DREBs), a cold stress-responsive gene, *COR15a*, a gene encoding the expression of a transcription factor involved in freezing and drought stress tolerance, *CAMTA1*, and transcription activators of ROS-related antioxidant genes, *ZAT10* and *ZAT12*, following cold stress (Bajwa *et al.*, 2014). The up-regulation of cold signalling genes by melatonin may stimulate the biosynthesis of compounds which protect the membrane structure against peroxidation during chilling. Melatonin has an important role in modulating the expression of a wide range of genes, reflecting its pleiotropic physiological roles in plants (Byeon *et al.*, 2013; Weeda *et al.*, 2014; Zhang *et al.*, 2014).

Metal exposure is associated with several toxic effects. Excess metals can interact with biological macromolecules causing oxidative damage. An adsorptive voltammetric study showed that melatonin and its precursors can bind to several toxic metals: aluminium with melatonin, tryptophan, and serotonin; cadmium with melatonin and tryptophan; copper with melatonin and serotonin; Fe<sup>3+</sup> with melatonin and serotonin; Fe<sup>2+</sup> with tryptophan only; lead with melatonin, tryptophan, and serotonin; and zinc with melatonin and tryptophan (Limson *et al.*, 1998). Electrochemical studies show that melatonin can bind to both Cu<sup>2+</sup> and Cu<sup>1+</sup> which cause free radical damage (Parmar *et al.*, 2002). It is documented that melatonin affects biological systems not only through direct quenching of free radicals but also via chelation of toxic metals (Flora *et al.*, 2013; Romero *et al.*, 2014).

## Conclusions and perspectives

Hundreds of articles relating to melatonin in plants have been published in the last 20 years. Research shows that melatonin plays critical roles in plant defence systems. Almost all the abiotic stresses which cause oxidative stress can be alleviated by melatonin treatment if the concentration of melatonin is appropriate. Researchers have demonstrated possible mechanisms of this protective effect.

However, there is still much that remains unknown and needs to be elucidated in the future. The precise location of melatonin biosynthesis in plants requires further investigation. Since melatonin is extremely labile, it is difficult to monitor the transport of melatonin in plant organelles and organs (van Tassel and O'Neill, 2001). Tan *et al.* (2013) mentioned that in most cells, mitochondria and chloroplasts may be the primary sites of melatonin synthesis. The root is perhaps the organ mentioned most frequently as the potential site of melatonin biosynthesis; however, evidence supporting this theory is limited and the unequivocal site of melatonin biosynthesis remains unknown. Melatonin has pleiotropic physiological roles in plants. Unfortunately, no specific phenotype associated with melatonin has been found in higher plants. Currently, no melatonin receptor(s) or binding site(s) have been identified in plants. In animals, various subtypes of receptors have been characterized, and their genes have been sequenced (Reppert, 1997; von Gall *et al.*, 2002). The possible presence of specific melatonin receptors in plant cells cannot be ruled out and is an important aspect in the study

of signal transduction of melatonin in plants. Analysis of the differential expression data generated by microarray and RNA-seq can offer new information for identifying putative melatonin receptor(s) in plants. Alternatively, since melatonin has a similar structure to auxin, it could possibly interact with the auxin receptor. In addition to melatonin, several isomers were identified in plants and plant products such as wine and bread (Vicente Gomez *et al.*, 2012; Vitalini *et al.* 2013; Yilmaz *et al.*, 2014). The study of melatonin isomers is an emerging area of research as they probably share many of the biological functions of melatonin (Tan *et al.*, 2012). It is another promising area of research to explore. All of this warrants further investigation in the near future.

The goal of this review is to update the reader on the roles of melatonin in plant abiotic stress resistance and to encourage plant scientists to investigate further the mechanism of melatonin-related resistance. We have summarized the biosynthesis, response to harsh conditions, roles in regulating stress resistance, and possible mechanisms. First of all, melatonin can be synthesized and taken up by plants. Genetic modification can enhance melatonin synthesis in transgenic plants and improve resistance to adverse conditions. Plants accumulate high levels of melatonin when faced with harsh environments. Exogenously applied melatonin helps improve tolerance to stresses. We also summarized the mechanisms of melatonin-induced abiotic stress resistance. In order to better understand the roles of this molecule and make full use of such roles, many more investigations must be conducted.

## Acknowledgements

This work was partly supported by the grant to Y.-D. Guo (2012AA101801, 2012CB113900, 2011BAD17B01). We appreciate all the help from National Energy R & D Center for Non-food Biomass and Beijing Key Laboratory of Growth and Developmental Regulation for Protected Vegetable Crops.

## References

- Afreen F, Zobayed S, Kozai T. 2006. Melatonin in *Glycyrrhiza uralensis*: response of plant roots to spectral quality of light and UV-B radiation. *Journal of Pineal Research* **41**, 108–115.
- Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. 2003. The chemistry of melatonin's interaction with reactive species. *Journal of Pineal Research* **34**, 1–10.
- Arnao MB, Hernández-Ruiz J. 2007. Melatonin promotes adventitious and lateral root regeneration in etiolated hypocotyls of *Lupinus albus* L. *Journal of Pineal Research* **42**, 147–152.
- Arnao MB, Hernández-Ruiz J. 2009a. Assessment of different sample processing procedures applied to the determination of melatonin in plants. *Phytochemical Analysis* **20**, 14–18.
- Arnao MB, Hernández-Ruiz J. 2009b. Chemical stress by different agents affects the melatonin content of barley roots. *Journal of Pineal Research* **46**, 295–299.
- Arnao MB, Hernández-Ruiz J. 2009c. Protective effect of melatonin against chlorophyll degradation during the senescence of barley leaves. *Journal of Pineal Research* **46**, 58–63.
- Arnao MB, Hernández-Ruiz J. 2006. The physiological function of melatonin in plants. *Plant Signaling and Behavior* **1**, 89–95.
- Arnao MB, Hernández-Ruiz J. 2013. Growth conditions determine different melatonin levels in *Lupinus albus* L. *Journal of Pineal Research* **55**, 149–155.
- Bajwa VS, Shukla MR, Sherif SM, Murch SJ, Saxena PK. 2014. Role of melatonin in alleviating cold stress in *Arabidopsis thaliana*. *Journal of Pineal Research* **56**, 238–245.
- Baxter A, Mittler R, Suzuki N. 2014. ROS as key players in plant stress signalling. *Journal of Experimental Botany* **65**, 1229–1240.
- Bose J, Rodrigo-Moreno A, Shabala S. 2014. ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of Experimental Botany* **65**, 1241–1257.
- Bouchereau A, Aziz A, Larher F, Martin-Tanguy J. 1999. Polyamines and environmental challenges: recent development. *Plant Science* **140**, 103–125.
- Byeon Y, Back KW. 2013. Melatonin synthesis in rice seedlings *in vivo* is enhanced at high temperatures and under dark conditions due to increased serotonin N-acetyltransferase and N-acetylserotonin methyltransferase activities. *Journal of Pineal Research* **56**, 189–195.
- Byeon Y, Back KW. 2014. An increase in melatonin in transgenic rice causes pleiotropic phenotypes, including enhanced seedling growth, delayed flowering, and low grain yield. *Journal of Pineal Research* **56**, 380–414.
- Byeon Y, Lee HY, Lee K, Park S, Back KW. 2014a. Cellular localization and kinetics of the rice melatonin biosynthetic enzymes SNAT and ASMT. *Journal of Pineal Research* **56**, 107–114.
- Byeon Y, Park S, Kim YS, Back KW. 2013. Microarray analysis of genes differentially expressed in melatonin-rich transgenic rice expressing a sheep serotonin N-acetyltransferase. *Journal of Pineal Research* **55**, 357–363.
- Byeon Y, Park S, Kim YS, Park DH, Lee S, Back KW. 2012. Light-regulated melatonin biosynthesis in rice during the senescence process in detached leaves. *Journal of Pineal Research* **53**, 107–111.
- Byeon Y, Park S, Lee HY, Kim YS, Back KW. 2014b. Elevated production of melatonin in transgenic rice seeds expressing rice tryptophan decarboxylase. *Journal of Pineal Research* **56**, 275–282.
- Chen GF, Huo YS, Tan DX, Liang Z, Zhang WB, Zhang YK. 2003. Melatonin in Chinese medicinal herbs. *Life Sciences* **73**, 19–26.
- Chen Q, Qi WB, Reiter RJ, Wei W, Wang BM. 2009. Exogenously applied melatonin stimulates root growth and raises endogenous indoleacetic acid in roots of etiolated seedlings of *Brassica juncea*. *Journal of Plant Physiology* **166**, 324–328.
- Cho MH, No HK, Prinyawiwatkul W. 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. *Journal of Food Science* **73**, 70–77.
- Cipolla-Neto J, Amaral FG, Afeche SC, Tan DX, Reiter RJ. 2014. Melatonin, energy metabolism, and obesity: a review. *Journal of Pineal Research* **56**, 371–381.
- De Luca V, Marineau C, Brisson N. 1989. Molecular-cloning and analysis of cDNA-encoding a plant tryptophan decarboxylase—comparison with animal dopa decarboxylases. *Proceedings of the National Academy of Sciences, USA* **86**, 2582–2586.
- Di Fiore S, Li QR, Leech MJ, Schuster F, Emans N, Fischer R, Schillberg S. 2002. Targeting tryptophan decarboxylase to selected subcellular compartments of tobacco plants affects enzyme stability and *in vivo* function and leads to a lesion-mimic phenotype. *Plant Physiology* **129**, 1160–1169.
- Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C, Schiwara HW, Schloot W. 1995. Melatonin in edible plants identified by radioimmunoassay and by high-performance liquid chromatography-mass spectrometry. *Journal of Pineal Research* **18**, 28–31.
- Flora SJS, Shrivastava R, Mittal M. 2013. Chemistry and pharmacological properties of some natural and synthetic antioxidants for heavy metal toxicity. *Current Medicinal Chemistry* **20**, 4540–4574.
- Foyer C, LopezDelgado H, Dat J, Scott I. 1997. Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signalling. *Physiologia Plantarum* **100**, 241–254.
- Foyer C, Noctor G. 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell and Environment* **28**, 1056–1071.
- Fujiwara T, Maisonneuve S, Isshiki M, Mizutani M, Chen L, Wong HL, Kawasaki T, Shimamoto K. 2010. *Sekiguchi lesion* gene encodes a cytochrome P450 monooxygenase that catalyzes conversion of tryptamine to serotonin in rice. *Journal of Biological Chemistry* **285**, 11308–11313.
- Hall J. 2002. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany* **53**, 1–11.



- Hernández-Ruiz J, Arnao MB.** 2008. Distribution of melatonin in different zones of lupin and barley plants at different ages in the presence and absence of light. *Journal of Agricultural and Food Chemistry* **56**, 10567–10573.
- Hernández-Ruiz J, Arnao MB.** 2008. Melatonin stimulates the expansion of etiolated lupin cotyledons. *Plant Growth Regulation* **55**, 29–34.
- Hernández-Ruiz J, Cano A, Arnao MB.** 2004. Melatonin: a growth-stimulating compound present in lupin tissues. *Planta* **220**, 140–144.
- Hernández-Ruiz J, Cano A, Arnao MB.** 2005. Melatonin acts as a growth-stimulating compound in some monocot species. *Journal of Pineal Research* **39**, 137–142.
- Hung S, Wang C, Ivanov S, Alexieva V, Yu C.** 2007. Repetition of hydrogen peroxide treatment induces a chilling tolerance comparable to cold acclimation in mung bean. *Journal of the American Society for Horticultural Science* **132**, 770–776.
- Jayaweera M, Kasturiarachchi J.** 2004. Removal of nitrogen and phosphorus from industrial wastewaters by phytoremediation using water hyacinth (*Eichhornia crassipes* (Mart.) Solms). *Water Science and Technology* **50**, 217–225.
- Kang K, Kim YS, Park S, Back KW.** 2009. Senescence-induced serotonin biosynthesis and its role in delaying senescence in rice leaves. *Plant Physiology* **150**, 1380–1393.
- Kang K, Kong K, Park S, Natsagdorj U, Kim Y, Back K.** 2011. Molecular cloning of a plant N-acetylserotonin methyltransferase and its expression characteristics in rice. *Journal of Pineal Research* **50**, 304–309.
- Kang K, Lee K, Park S, Byeon Y, Back KW.** 2013. Molecular cloning of rice serotonin N-acetyltransferase, the penultimate gene in plant melatonin biosynthesis. *Journal of Pineal Research* **55**, 7–13.
- Kang K, Lee K, Park S, Kim YS, Back KW.** 2010. Enhanced production of melatonin by ectopic overexpression of human serotonin N-acetyltransferase plays a role in cold resistance in transgenic rice seedlings. *Journal of Pineal Research* **49**, 176–182.
- Kang S, Kang K, Lee K, Back KW.** 2007. Characterization of tryptamine 5-hydroxylase and serotonin synthesis in rice plants. *Plant Cell Reports* **26**, 2009–2015.
- Kang S, Kang K, Lee K, Back KW.** 2008. Characterization of rice tryptophan decarboxylases and their direct involvement in serotonin biosynthesis in transgenic rice. *Planta* **227**, 263–272.
- Kanjanaphachot P, Wei BY, Lo SF, Wang IW, Wang CS, Yu SM, Yen ML, Chiu SH, Lai CC, Chen L.** 2012. Serotonin accumulation in transgenic rice by over-expressing tryptophan decarboxylase results in a dark brown phenotype and stunted growth. *Plant Molecular Biology* **78**, 525–543.
- Kim T, Kim S, Han T, Lee J, Chang S.** 2002. ABA and polyamines act independently in primary leaves of cold-stressed tomato (*Lycopersicon esculentum*). *Physiologia Plantarum* **115**, 370–376.
- Kolar J, Johnson C, Machackova I.** 2003. Exogenously applied melatonin (N-acetyl-5-methoxytryptamine) affects flowering of the short-day plant *Chenopodium rubrum*. *Physiologia Plantarum* **118**, 605–612.
- Koyama FC, Carvalho TLG, Alves E, da Silva HB, de Azevedo MF, Hemery AS, Garcia CRS.** 2013. The structurally related auxin and melatonin tryptophan-derivatives and their roles in *Arabidopsis thaliana* and in the human malaria parasite *Plasmodium falciparum*. *Journal of Eukaryotic Microbiology* **60**, 646–651.
- Kratsch H, Wise R.** 2000. The ultrastructure of chilling stress. *Plant, Cell and Environment* **23**, 337–350.
- Lei X, Zhu R, Zhang G, Dai Y.** 2004. Attenuation of cold-induced apoptosis by exogenous melatonin in carrot suspension cells: the possible involvement of polyamines. *Journal of Pineal Research* **36**, 126–131.
- Lerner AB, Case J, Takahashi Y, Lee T, Mori W.** 1958. Isolation of melatonin, the pineal gland factor that lightens melanocytes. *Journal of the American Chemical Society* **80**, 2587–2587.
- Li C, Wang P, Wei Z, Liang D, Liu C, Yin L, Jia D, Fu M, Ma F.** 2012. The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *Journal of Pineal Research* **53**, 298–306.
- Limson J, Nyokong T, Daya S.** 1998. The interaction of melatonin and its precursors with aluminium, cadmium, copper, iron, lead, and zinc: an adsorptive voltammetric study. *Journal of Pineal Research* **24**, 15–21.
- Manchester LC, Tan DX, Reiter RJ, Park W, Monis K, Qi W.** 2000. High levels of melatonin in the seeds of edible plants—possible function in germ tissue protection. *Life Sciences* **67**, 3023–3029.
- Misbahuddin M, Fariduddin A.** 2002. Water hyacinth removes arsenic from arsenic-contaminated drinking water. *Archives of Environmental Health* **57**, 516–518.
- Mittler R.** 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* **7**, 405–410.
- Moschou PN, Roubelakis-Angelakis KA.** 2014. Polyamines and programmed cell death. *Journal of Experimental Botany* **65**, 1285–1296.
- Munavalli GR, Saler PS.** 2009. Treatment of dairy wastewater by water hyacinth. *Water Science and Technology* **59**, 713–722.
- Murch SJ, Campbell SSB, Saxena PK.** 2001. The role of serotonin and melatonin in plant morphogenesis: regulation of auxin-induced root organogenesis in *in vitro*-cultured explants of St. John's wort (*Hypericum perforatum* L.). *In Vitro Cellular and Developmental Biology-Plant* **37**, 786–793.
- Murch SJ, KrishnaRaj S, Saxena P.** 2000. Tryptophan is a precursor for melatonin and serotonin biosynthesis in *in vitro* regenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. *Plant Cell Reports* **19**, 698–704.
- Okazaki M, Higuchi K, Hanawa Y, Shiraiwa Y, Ezura H.** 2009. Cloning and characterization of a *Chlamydomonas reinhardtii* cDNA arylalkylamine N-acetyltransferase and its use in the genetic engineering of melatonin content in the Micro-Tom tomato. *Journal of Pineal Research* **46**, 373–382.
- Paredes SD, Korkmaz A, Manchester LC, Tan DX, Reiter RJ.** 2009. Phytomelatonin: a review. *Journal of Experimental Botany* **60**, 57–69.
- Park S, Back KW.** 2012. Melatonin promotes seminal root elongation and root growth in transgenic rice after germination. *Journal of Pineal Research* **53**, 385–389.
- Park S, Byeon Y, Back KW.** 2013a. Functional analyses of three ASMT gene family members in rice plants. *Journal of Pineal Research* **55**, 409–415.
- Park S, Kang K, Lee K, Choi D, Kim Y, Back K.** 2009. Induction of serotonin biosynthesis is uncoupled from the coordinated induction of tryptophan biosynthesis in pepper fruits (*Capsicum annuum*) upon pathogen infection. *Planta* **230**, 1197–1206.
- Park S, Lee DE, Jang H, Byeon Y, Kim YS, Back K.** 2013b. Melatonin-rich transgenic rice plants exhibit resistance to herbicide-induced oxidative stress. *Journal of Pineal Research* **54**, 258–263.
- Parmar P, Limson J, Nyokong T, Daya S.** 2002. Melatonin protects against copper-mediated free radical damage. *Journal of Pineal Research* **32**, 237–242.
- Passaia G, Queval G, Bai J, Margis-Pinheiro M, Foyer CH.** 2014. The effects of redox controls mediated by glutathione peroxidases on root architecture in *Arabidopsis thaliana*. *Journal of Experimental Botany* **65**, 1403–1413.
- Pelagio-Flores R, Munoz-Parra E, Ortiz-Castro R, Lopez-Bucio J.** 2012. Melatonin regulates *Arabidopsis* root system architecture likely acting independently of auxin signaling. *Journal of Pineal Research* **53**, 279–288.
- Posmyk M, Balabusta M, Wieczorek M, Sliwinska E, Janas K.** 2009. Melatonin applied to cucumber (*Cucumis sativus* L.) seeds improves germination during chilling stress. *Journal of Pineal Research* **46**, 214–223.
- Posmyk MM, Janas KM.** 2009. Melatonin in plants. *Acta Physiologiae Plantarum* **31**, 1–11.
- Posmyk MM, Kuran H, Marciniak K, Janas KM.** 2008. Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. *Journal of Pineal Research* **45**, 24–31.
- Prasad T, Anderson M, Martin B, Stewart C.** 1994. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen-peroxide. *The Plant Cell* **6**, 65–74.
- Reiter RJ, Tan D-X, Fuentes-Broto L.** 2010. Melatonin: a multitasking molecule. *Progress in Brain Research* **181**, 127–151.
- Reppert S.** 1997. Melatonin receptors: molecular biology of a new family of G protein-coupled receptors. *Journal of Biological Rhythms* **12**, 528–531.
- Riddle S, Tran H, Dewitt J, Andrews J.** 2002. Field, laboratory, and X-ray absorption spectroscopic studies of mercury accumulation by water hyacinths. *Environmental Science and Technology* **36**, 1965–1970.

- Riga P, Medina S, Garcia-Flores LA, Gil-Izquierdo A.** 2014. Melatonin content of pepper and tomato fruits: effects of cultivar and solar radiation. *Food Chemistry* **156**, 347–352.
- Rodriguez C, Mayo J, Sainz R, Antolin I, Herrera F, Martin V, Reiter RJ.** 2004. Regulation of antioxidant enzymes: a significant role for melatonin. *Journal of Pineal Research* **36**, 1–9.
- Romero A, Ramos E, de Los Ríos C, Egea J, del Pino J, Reiter RJ.** 2014. A review of metal-catalyzed molecular damage: protection by melatonin. *Journal of Pineal Research* **56**, 343–370.
- Sarropoulou VN, Dimassi-Theriu K, Therios I, Koukourikou-Petridou M.** 2012a. Melatonin enhances root regeneration, photosynthetic pigments, biomass, total carbohydrates and proline content in the cherry rootstock PHL-C (*Prunus avium* × *Prunus cerasus*). *Plant Physiology and Biochemistry* **61**, 162–168.
- Sarropoulou VN, Therios IN, Dimassi-Theriu K.** 2012b. Melatonin promotes adventitious root regeneration in *in vitro* shoot tip explants of the commercial sweet cherry rootstocks CAB-6P (*Prunus cerasus* L.), Gisela 6 (*P. cerasus* × *P. canescens*), and MxM 60 (*P. avium* × *P. mahaleb*). *Journal of Pineal Research* **52**, 38–46.
- Sarrou E, Therios I, Dimassi-Theriu K.** 2014. Melatonin and other factors that promote rooting and sprouting of shoot cuttings in *Punica granatum* cv. Wonderful. *Turkish Journal of Botany* **38**, 293–301.
- Shida C, Castrucci A, Lamyfreund M.** 1994. High melatonin solubility in aqueous-medium. *Journal of Pineal Research* **16**, 198–201.
- Simopoulos A, Tan DX, Manchester LC, Reiter RJ.** 2005. Purslane: a plant source of omega-3 fatty acids and melatonin. *Journal of Pineal Research* **39**, 331–332.
- Singhal V, Rai J.** 2003. Biogas production from water hyacinth and channel grass used for phytoremediation of industrial effluents. *Bioresource Technology* **86**, 221–225.
- Szafrańska K, Glinska S, Janas KM.** 2012. Changes in the nature of phenolic deposits after re-warming as a result of melatonin pre-sowing treatment of *Vigna radiata* seeds. *Journal of Plant Physiology* **169**, 34–40.
- Szafrańska K, Glinska S, Janas KM.** 2013. Ameliorative effect of melatonin on meristematic cells of chilled and re-warmed *Vigna radiata* roots. *Biologia Plantarum* **57**, 91–96.
- Tal O, Haim A, Harel O, Gerchman Y.** 2011. Melatonin as an antioxidant and its semi-lunar rhythm in green macroalgae *Ulva* sp. *Journal of Experimental Botany* **62**, 1903–1910.
- Tan DX, Hardeland R, Manchester LC, Rosales-Corral S, Coto-Montes A, Boga JA, Reiter RJ.** 2012. Emergence of naturally occurring melatonin isomers and their proposed nomenclature. *Journal of Pineal Research* **53**, 113–121.
- Tan DX, Manchester LC, Di Mascio P, Martinez GR, Prado FM, Reiter RJ.** 2007a. Novel rhythms of N-1-acetyl-N-2-formyl-5-methoxykynuramine and its precursor melatonin in water hyacinth: importance for phytoremediation. *FASEB Journal* **21**, 1724–1729.
- Tan DX, Manchester LC, Helton P, Reiter RJ.** 2007b. Phytoremediative capacity of plants enriched with melatonin. *Plant Signaling and Behavior* **2**, 514–516.
- Tan DX, Manchester LC, Liu XY, Rosales-Corral SA, Acuna-Castroviejo D, Reiter RJ.** 2013. Mitochondria and chloroplasts as the original sites of melatonin synthesis: a hypothesis related to melatonin's primary function and evolution in eukaryotes. *Journal of Pineal Research* **54**, 127–138.
- Tan DX, Manchester LC, Reiter RJ, Plummer B, Limson J, Weintraub S, Qi W.** 2000. Melatonin directly scavenges hydrogen peroxide: a potentially new metabolic pathway of melatonin biotransformation. *Free Radical Biology and Medicine* **29**, 1177–1185.
- Tiryaki I, Keles H.** 2012. Reversal of the inhibitory effect of light and high temperature on germination of *Phacelia tanacetifolia* seeds by melatonin. *Journal of Pineal Research* **52**, 332–339.
- Trivedy R, Pattanshetty S.** 2002. Treatment of dairy waste by using water hyacinth. *Water Science and Technology* **45**, 329–334.
- Uchendu EE, Shukla MR, Reed BM, Saxena PK.** 2013. Melatonin enhances the recovery of cryopreserved shoot tips of American elm (*Ulmus americana* L.). *Journal of Pineal Research* **55**, 435–442.
- van Tassel D, O'Neill S.** 2001. Putative regulatory molecules in plants: evaluating melatonin. *Journal of Pineal Research* **31**, 1–7.
- Vicente Gomez FJ, Raba J, Cerutti S, Fernanda Silva M.** 2012. Monitoring melatonin and its isomer in *Vitis vinifera* cv. Malbec by UHPLC-MS/MS from grape to bottle. *Journal of Pineal Research* **52**, 349–355.
- Vitalini S, Gardana C, Simonetti P, Fico G, Iriti M.** 2013. Melatonin, melatonin isomers and stilbenes in Italian traditional grape products and their antiradical capacity. *Journal of Pineal Research* **54**, 322–333.
- von Gall C, Stehle J, Weaver D.** 2002. Mammalian melatonin receptors: molecular biology and signal transduction. *Cell and Tissue Research* **309**, 151–162.
- Wang H, Li L, Zhao M, Chen YH, Zhang ZH, Zhang C, Ji YL, Meng XH, Xu DX.** 2011. Melatonin alleviates lipopolysaccharide-induced placental cellular stress response in mice. *Journal of Pineal Research* **50**, 418–426.
- Wang L, Zhao Y, Reiter RJ, He C, Liu G, Lei Q, Zuo B, Zheng XD, Li Q, Kong J.** 2014. Changes in melatonin levels in transgenic 'Micro-Tom' tomato overexpressing ovine AANAT and ovine HIOMT genes. *Journal of Pineal Research* **56**, 134–142.
- Wang P, Sun X, Chang C, Feng F, Liang D, Cheng L, Ma FW.** 2013a. Delay in leaf senescence of *Malus hupehensis* by long-term melatonin application is associated with its regulation of metabolic status and protein degradation. *Journal of Pineal Research* **55**, 424–434.
- Wang P, Sun X, Li C, Wei Z, Liang D, Ma FW.** 2013b. Long-term exogenous application of melatonin delays drought-induced leaf senescence in apple. *Journal of Pineal Research* **54**, 292–302.
- Wang P, Yin L, Liang D, Li C, Ma F, Yue Z.** 2012. Delayed senescence of apple leaves by exogenous melatonin treatment: toward regulating the ascorbate–glutathione cycle. *Journal of Pineal Research* **53**, 11–20.
- Weeda S, Zhang N, Zhao X, Ndiop G, Guo Y, Buck GA, Fu C, Ren SX.** 2014. Arabidopsis transcriptome analysis reveals key roles of melatonin in plant defense systems. *PLoS One* **9**, e93462–e93462.
- Xia H, Ma X.** 2006. Phytoremediation of ethion by water hyacinth (*Eichhornia crassipes*) from water. *Bioresource Technology* **97**, 1050–1054.
- Yilmaz C, Kocadagli T, Gokmen V.** 2014. Formation of melatonin and its isomer during bread dough fermentation and effect of baking. *Journal of Agricultural and Food Chemistry* **62**, 2900–2905.
- Yin L, Wang P, Li M, et al.** 2013. Exogenous melatonin improves *Malus* resistance to Marssonina apple blotch. *Journal of Pineal Research* **54**, 426–434.
- Zhang HX, Blumwald E.** 2001. Transgenic salt-tolerant tomato plants accumulate salt in foliage but not in fruit. *Nature Biotechnology* **19**, 765–768.
- Zhang L, Jia J, Xu Y, Wang Y, Hao J, Li T.** 2012. Production of transgenic *Nicotiana glauca* plants expressing melatonin synthetase genes and their effect on UV-B-induced DNA damage. *In Vitro Cellular and Developmental Biology-Plant* **48**, 275–282.
- Zhang N, Zhang HJ, Zhao B, et al.** 2014. The RNA-seq approach to discriminate gene expression profiles in response to melatonin on cucumber lateral root formation. *Journal of Pineal Research* **56**, 39–50.
- Zhang N, Zhao B, Zhang HJ, Weeda S, Yang C, Yang ZC, Ren SX, Guo YD.** 2013. Melatonin promotes water-stress tolerance, lateral root formation, and seed germination in cucumber (*Cucumis sativus* L.). *Journal of Pineal Research* **54**, 15–23.
- Zhao Y, Qi L, Wang W, Saxena P, Liu C.** 2011. Melatonin improves the survival of cryopreserved callus of *Rhodiola crenulata*. *Journal of Pineal Research* **50**, 83–88.